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The clinical potential of proteasome inhibition

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Abstract

The development of the proteasome inhibitor bortezomib is a prime example of successful bench-to-bedside research. NF-κB is implicated in the pathogenesis of multiple myeloma (MM), and bortezomib blocks NF-κB activation and its sequelae. Additional bortezomib mechanisms include interactions at the MM cell surface and mitochondrial level and in downstream apoptotic signaling. In the pivotal bortezomib study, the response rate was 35%; time to progression (TTP), 7 months; and median duration of response, 14.3 months. Responses were associated with improvements in quality of life, increases in laboratory parameters, and decreased transfusions. Bortezomib is thus indicated in the US for patients with relapsed, progressive MM and recommended for approval in Europe. The confirmatory phase III trial has been stopped early due to a significant improvement in TTP with bortezomib. The success of bortezomib in MM demonstrates the utility of targeting the tumour:host interaction and BM milieu, in addition to the tumour itself.

Keywords: Proteasome; Bortezomib; Multiple myeloma; NF-κB; Boronic acids

The story of the proteasome inhibitor bortezomib (VELCADE) in multiple myeloma (MM) is a wonderful example of translational research. In 1994, Dharminder Chauhan in our laboratory showed that the binding of MM cells to bone marrow stromal cells (BMSCs) triggered interleukin-6 (IL-6) transcription and secretion in an NF-κB-dependent mechanism [1,2]. Others have shown that NF-κB conferred drug resistance in MM cells [3] and that adhesion molecules mediating binding of MM cells to extracellular matrix proteins [4] and to BMSCs was also NF-κB dependent [5].

Investigators discovered that proteasomes, multisubunit catalytic proteins with a 20S core symmetrically bound to two 19S regulatory caps, are responsible for the majority of protein degradation in cells. The proteasome is part of the ubiquitin–proteasome pathway which plays an essential role in the degradation of proteins that are relevant to cancer initiation and progression including cell cycle regulators (p21, p27, and cyclin E), pro- and anti-apoptosis factors (Bcl-2, Bax), and inhibitory proteins such as inhibitor-kappaB (IκB). The ordered ubiquitination and degradation of these proteins is required for the cell to progress through the cell cycle and undergo mitosis. Inhibition of the proteasome leads to cell cycle arrest in normal cells and apoptosis in transformed cells. The proteasome inhibitor bortezomib is a modified dipeptidyl boronic acid that binds selectively and reversibly to the proteasome and inhibits chymotryptic-like activity within the β ring of the 26S proteasome. By inhibiting the proteasome, bortezomib affects multiple signaling pathways and the antineoplastic activity involves inhibition of cell growth and survival, induction of apoptosis, and inhibition of expression of genes which control cellular adhesion, migration, and angiogenesis.

One of these actions is the inhibition of NF-κB activation [6] (Fig. 1). Initially this was not pursued because of concerns that inhibiting NF-κB would cause nonspecific toxicity. As the body of evidence on the specificity of bortezomib in transformed cells and its ability to induce apoptosis grew, Millennium Pharmaceuticals, Inc. began phase I studies that showed bortezomib was well tolerated and had early signs of anti-MM activity; of the first nine patients, one achieved a complete

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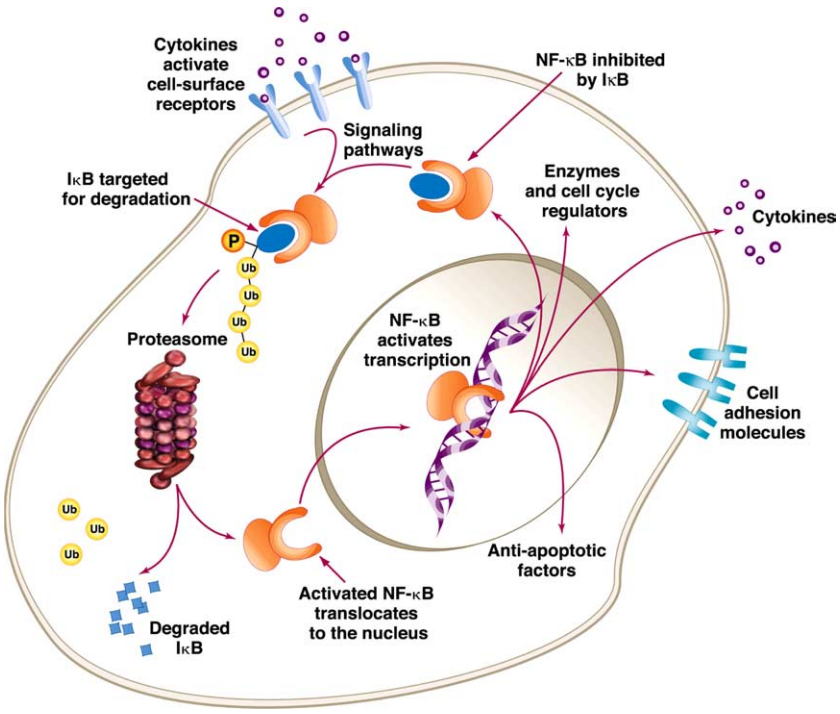

Fig. 1. The inhibition of NF-κB activation.

response, and a decrease in M-protein levels or stable disease was achieved in eight patients [7].

In 1999 our laboratory conducted *in vitro* studies that showed that bortezomib induced apoptosis even of drug-resistant MM cell lines and patient cells and that it overcame the resistance to conventional chemotherapy conferred both by binding of MM cells to extracellular matrix proteins and BMSCs, as well as by the related induction and secretion of cytokines such as IL-6 [8]. We then went on to show that bortezomib also inhibited growth of human MM cells in a severe combined immune deficiency (SCID) mouse model [9].

Very rapidly thereafter in 2001 Dr. Paul Richardson in our center chaired a multicenter phase II trial of bortezomib in refractory relapsed MM which showed 35% response rates (including 4% complete responses); response duration of 14.3 months and survival of 17.8 months versus the 6–9 month expected survival; and associated clinical benefit including increased haemoglobin levels and decreased transfusion requirements, improved quality of life, stabilisation or improvement in renal function, and improvement in suppressed normal immunoglobulins [10]. On the basis of these results, VELCADE was approved for use in the United States in May 2003 and recently recommended for approval in Europe for patients with MM who have received at least 2 prior therapies, with disease progression noted on the last therapy.

A confirmatory phase III study of VELCADE versus high-dose dexamethasone in relapsed/refractory patients recently completed accrual. Of note, in December 2003, the dexamethasone control arm was halted on the recommendation of the data safety monitoring committee due to a significant improvement in time to progression, the primary endpoint, with VELCADE.

Gene microarray and proteomic analyses are both determining targets of resistance and sensitivity to bortezomib and providing the preclinical rationale for clinical protocols combining bortezomib with conventional and other novel therapies [11–13].

As noted above, the rationale for use of bortezomib in MM was inhibition of NF-κB not only in the MM cell but also in the BM milieu. Myeloma cells migrate to the BM microenvironment and there bind to extracellular matrix proteins and BMSCs [14], which not only localises them in the BM but also confers cell adhesion-related drug resistance and triggers transcription and secretion of cytokines [IL-6, vascular endothelial growth factor (VEGF), insulin-like growth factor-1 (IGF-1)] [2,15,16]. Both the expression of adhesion molecules on MM cells and BMSCs as well as the constitutive and MM binding triggered transcription of these cytokines are modulated by NF-κB. Inhibiting activation of NF-κB was therefore the original rationale for use of the proteasome inhibitor bortezomib. Specifically, NF-κB is constitutively in its inactive state in the cytosol due to binding to IκB inhibitor protein. This protein, as most intracellular proteins, is ubiquitinated and then targeted for breakdown by the proteasome. Once degraded, NF-κB can then translocate to the nucleus, bind to the appropriate motif, and induce transcription of target genes. Bortezomib blocks

the degradation of IκB, thereby keeping NF-κB in its inactivated state.

Our studies have shown that bortezomib targets the MM cell, the tumour cell–host interaction, and the BM milieu. Specifically, bortezomib induces apoptosis of drug resistant MM cell lines and patient cells, including tumour cells resistant to alkylating agents, anthracyclines, and steroids [8]. In addition, it downregulates expression of adhesion molecules on tumour cells and BMSCs, thereby blocking adhesion of MM cells to BM [5]. It inhibits both intrinsic and MM cell binding-induced transcription and secretion of cytokines mediating MM cell growth, survival, and drug resistance [8]. Finally it also inhibits angiogenesis.

Having shown *in vitro* ability of bortezomib to overcome conventional drug resistance, we next tested its *in vivo* activity against human MM cells in a SCID mouse model [9]. Human MM cells were injected subcutaneously into SCID mice which were then treated with either saline control or bortezomib. Daily treatment with either 0.5 or 1.0 mg/kg drug significantly reduced tumour volume and prolonged survival. In this model, biopsy of the tumour revealed apoptosis and increased sub G0/G1 cells in the bortezomib-treated cohort compared to the control saline treated animals in whom tumour remained viable, with few apoptotic cells. Tumour-associated angiogenesis was also inhibited in this model.

Although the original premise for the use of bortezomib in MM was inhibition of NF-κB, we have shown that it has multiple other actions. First, we compared the effect of treatment with the specific NF-κB inhibitor, IκB kinase inhibitor PS-1145, versus bortezomib on MM cell growth [17]. The IκK inhibitor PS-1145 had little, if any, effect on MM cell proliferation, whereas 0.01 mM bortezomib markedly inhibited growth. This suggests that bortezomib must be acting directly on MM cells in ways other than NF-κB inhibition. Of note, PS-1145 does inhibit the proliferation, of MM cells adherent to BMSCs, as well as both the constitutive and MM binding-induced transcription and secretion of IL-6.

We next examined effects of bortezomib treatment of MM cells at the levels of the cell surface, mitochondria, and downstream caspase signaling cascades. Bortezomib induces degradation of gp130, the J component of the IL-6 receptor, at early time points while MM cells are still viable, suggesting that part of the selectivity of this agent for MM cells may be related to lack of responsiveness to IL-6 and associated growth, survival, and drug resistance [18]. We have shown that bortezomib treatment of MM cells triggers activation of JNK, with release of proapoptotic second mitochondrial activator of caspases (Smac) and cytochrome-*c* and activation of downstream caspases [19]. It also induces generation of reactive oxygenation species, with associated release of Smac and cytochrome-*c* [20]. Finally and most importantly, examining the pattern of triggering of apoptotic signaling cascades triggered by conventional and novel therapies not only defines their mechanism of action, but also provides the preclinical rationale for combining novel agents with conventional agents or each other in clinical treatment protocols [21]. For example, one can combine agents which both trigger a single cascade, i.e. Revimid and TRAIL inducing caspase 8, or combine agents which trigger distinct cascades, i.e. Revimid and bortezomib inducing caspase 8 and caspase 9 [22].

Finally we are utilizing gene microarray profiling and proteomics both to define molecular targets of sensitivity and resistance to novel agents and to provide the rationale for their clinical application. For example, exposure of MM cells to bortezomib induces caspase, downregulates survival, and upregulates heat shock protein (hsp) stress response 90 transcripts [12]. This data provided the rationale for exposing MM cells to bortezomib and then blocking the induction of hsp90 with 17 AAG. A clinical trial of this combination will begin soon, based upon the significantly increased MM cell cytotoxicity observed *in vitro*. Analysis of protein expression reveals that bortezomib induces cleavage of DNA repair kinases, i.e. DNA-PKcs, the first evidence that the proteasome inhibitor bortezomib inhibits DNA repair [13]. Bortezomib can enhance sensitivity and overcome resistance to DNA damaging agents *in vitro*, and already early clinical experience suggests that addition of bortezomib can overcome clinical resistance to melphalan and doxil. Therefore gene array and proteomic analyses will allow for the rational clinical evaluation of bortezomib together with conventional and other novel therapies. Such application of translational research to the clinic underscores the continued important role of proteasome inhibitors in the treatment of multiple myeloma and ultimately, perhaps, across a broad spectrum of malignancies.

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